RESEARCH PAPER

Positive root pressure is critical for whole-plant desiccation recovery in two species of terrestrial resurrection ferns

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Abstract

Desiccation-tolerant (DT) organisms can lose nearly all their water without dying. Desiccation tolerance allows organisms to survive in a nearly completely dehydrated, dormant state. At the cellular level, sugars and proteins stabilize cellular components and protect them from oxidative damage. However, there are few studies of the dynamics and drivers of whole-plant recovery in vascular DT plants. In vascular DT plants, whole-plant desiccation recovery (resurrection) depends not only on cellular rehydration, but also on the recovery of organs with unequal access to water. In this study, *in situ* natural and artificial irrigation experiments revealed the dynamics of desiccation recovery in two DT fern species. Organ-specific irrigation experiments revealed that the entire plant resurrected when water was supplied to roots, but leaf hydration alone (foliar water uptake) was insufficient to rehydrate the stele and roots. In both species, pressure applied to petioles of excised desiccated fronds resurrected distal leaf tissue, while capillarity alone was insufficient to resurrect distal pinnules. Upon rehydration, sucrose levels in the rhizome and stele dropped dramatically as starch levels rose, consistent with the role of accumulated sucrose as a desiccation protectant. These findings provide insight into traits that facilitate desiccation recovery in dryland ferns associated with chaparral vegetation of southern California.

Keywords: Drought, foliar water uptake, F_v/F_m, non-structural carbohydrates, *Pellaea andromedifolia*, *Pentagramma triangularis*, plant rehydration.

Introduction

Desiccation tolerance describes an organism's ability to dry to equilibrium with the air and then recover metabolic activity upon rehydration. Functionally, this means that desiccation–tolerant (DT) organisms can tolerate the loss of most of their water (10% water content or −100 MPa; Alpert, 2005). The evolution of desiccation tolerance probably allowed early plants to colonize land before stomata and a vascular system regulated water loss (Gaff and Oliver, 2013). Desiccation tolerance protects most bryophytes from frequent dry periods (reviewed in Proctor *et al*., 2007). Almost all vascular plants have retained desiccation tolerance in the seed or spore stage, but a few ferns and angiosperms have re-evolved desiccation tolerance in their

leaves, stems, or roots (Oliver *et al.*, 2000; Gaff and Oliver, 2013; VanBuren *et al.*, 2017). Plants that express desiccation tolerance in these vegetative tissues are colloquially referred to as resurrection plants. Vascular resurrection plants thrive in seasonally dry regions where desiccation-sensitive plants have lower survival rates and fitness, and they are most common on sandy slopes and rocky outcrops (Gaff, 1977, 1987; Porembski and Barthlott, 2000; Alcantara *et al.*, 2015).

Cellular recovery from near-complete dehydration is complex and requires the coordination of physical and chemical processes. The desiccated state presents at least three challenges at the cellular level: photo-oxidative stress caused by reactive oxygen species, the metabolic requirements of resurrection, and the mechanical stress of cell and tissue deformation (Farrant *et al.*, 2007). While desiccated, the cellular components are usually stabilized by protective sugars and proteins, forming a 'glassy' state (Moore *et al.*, 2007; Peters *et al.*, 2007). This glassy state reduces the damage caused by reactive oxygen species, and the sugars may also provide energy to fuel metabolic processes during rehydration (Scott, 2000). Mechanical stress is alleviated by tissue structures that facilitate cell folding and prevent extensive tissue damage (Moore *et al.*, 2007).

Most DT organisms are relatively small and composed of only one or a few cell layers (e.g. algae, lichens, bryophytes, and fern gametophytes), making rehydration a relatively simple process. When water first contacts these desiccated tissues, it easily spreads on the surface or through adjoining cells (Proctor, 2000; Proctor *et al.*, 2007). However, vascular DT plants such as ferns and angiosperms face the added challenge of resurrecting distinct tissues and organs with unequal access to water, adding another layer of complexity to whole-plant recovery. For instance, the roots of a desiccated vascular plant have a reliable water source after rain (wet soil) for days or weeks, but the leaf surfaces may be subject to ephemeral wetting and high atmospheric water demand, probably drying within a day following a rainfall event. Although previous studies have tried to elucidate the mechanism of whole-plant recovery in a DT angiosperm, *Myrothamnus flabellifolius* (Sherwin *et al.*, 1998; Schneider *et al.*, 2000), these studies found contradictory results regarding the role of root pressure in whole-plant recovery of this species. Furthermore, this question remains untested in DT ferns. Ferns and angiosperms have contrasting hydraulic anatomy and may show different patterns of whole-plant recovery.

Several species of resurrection ferns thrive in the rocky soils of the California chaparral ecosystem, including locally abundant *Pellaea andromedifolia* and *Pentagramma triangularis*. These plants have small stature, with fronds rarely exceeding 50 cm and 20 cm in height, respectively. Their rhizomes are typically found 2 cm below the soil surface, and their roots are fibrous and shallow (Holmlund *et al.*, 2016). These two species co-occur in the rocky outcrops and shallow soil characteristic of exposed chaparral slopes, and they grow in niches with higher irradiance compared with other chaparral fern species (Holmlund *et al.*, 2016). The southern California chaparral ecosystem is subject to long summer droughts (Cowling *et al.*, 1996). During drought, these two DT species completely desiccate but normally resurrect within several days of the first rain event. However, we observed that only the smallest fronds

or basal pinnules (leaflets) resurrected following a small rain event (5 mm, 28 February 2014; Fig. 1); more precisely, only the leaf tissue closest to the root water source resurrected. This observation suggested that water from the roots contributed more to whole-plant recovery than did leaf water uptake. In contrast, if leaf water uptake were the primary water source for resurrected ferns, then all fronds or pinnules would have resurrected equally following a small rain event.

Consequently, we hypothesized that these ferns resurrect via bottom-up rehydration (root water uptake) rather than top-down rehydration (leaf water uptake). We tested this hypothesis using three sets of natural and artificial irrigation treatments on desiccated plants by quantifying recovery in the root, stele, and leaf tissue (Fig. 2). First, we quantified desiccation recovery following a natural rain event in which we excluded water from the leaves of some plants *in situ*. This experiment demonstrated that root water alone was sufficient to resurrect all parts of the plant. Next, we used organ-specific *in situ* irrigation experiments during the dry season to determine if leaf water uptake alone can resurrect the whole plant. Pilot data revealed that these resurrection ferns consistently generated root pressure during the early stages of recovery. Thus, we suspected that positive root pressure rather than capillary action alone is necessary for whole-plant recovery. We tested this hypothesis by resurrecting excised fronds using either simulated root pressure or capillary action alone.

Additionally, we theorized that changes in non-structural carbohydrate (NSC) content in the leaves, stipe vascular bundles (stele), and rhizomes might provide further insight into the desiccation and recovery dynamics in these ferns. Therefore, we tracked the NSC content of leaves, steles, and rhizomes in *P. andromedifolia* during recovery following a natural rain event. Since sucrose is a known desiccation protectant, we hypothesized that all organs would have high sucrose concentrations per unit dry weight in the desiccated state. Taken together, our
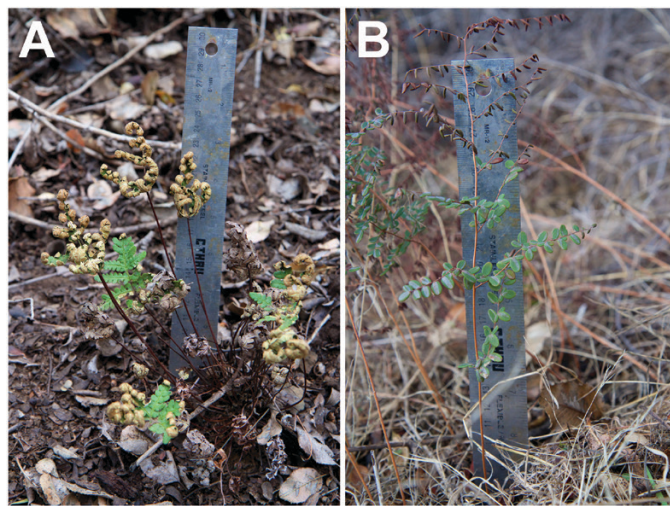

Fig. 1. Two partially resurrected chaparral ferns following 5 mm rainfall in February 2014. (A) Only the shorter fronds (uncurled) of this *Pentagramma triangularis* individual have resurrected, while the taller fronds remain desiccated (curled). (B) Only the lower pinnules (leaflets) of this *Pellaea andromedifolia* frond have resurrected (uncurled), while the upper pinnules remain desiccated (curled). (This figure is available in color at *JXB* online.)
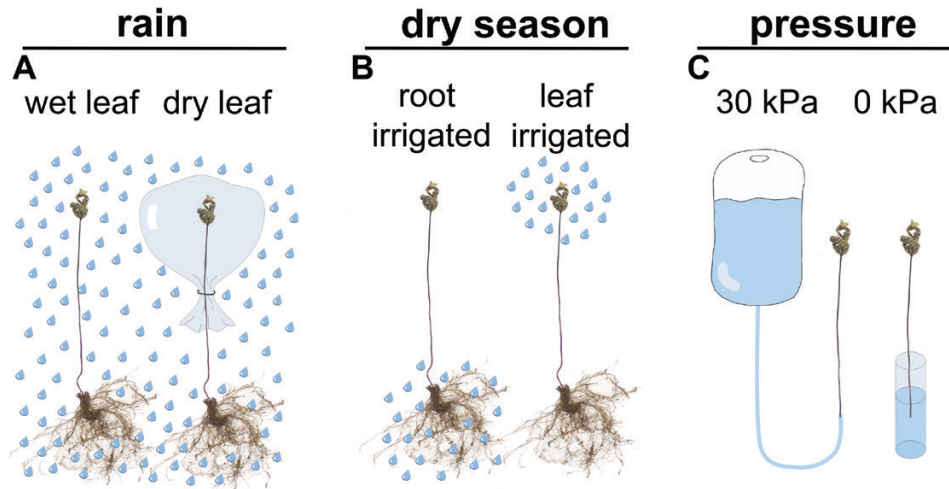
Fig. 2. Illustration of three separate experiments used in this study: a natural rain event with bagged and unbagged fronds, dry season root and leaf irrigation, and growth chamber experiments on excised fronds either pressurized or non-pressurized at the cut end of their stipe. (A) The first rain event of the wet season in the Santa Monica Mountains occurred on 8–9 January 2018. During this rain event, the leaves of some plants were covered (dry leaf treatment), while some leaves were left exposed (wet leaf control). All roots were completely soaked by the rain. (B) At the end of the dry season (September–October 2017), desiccated plants were irrigated *in situ* at our field sites. The roots of individuals in the root irrigation treatment were watered daily (9.5 liters; days 0–15 for a total of 152 liters each over 16 d). The leaves of individuals in the leaf irrigation treatment were kept wet continually by spraying with de-ionized water. (C) To determine the importance of applied water pressure to the resurrection of desiccated fronds, excised fronds were resurrected in a growth chamber using either 30 kPa of applied water pressure to the cut end of the frond or capillary action alone (0 kPa control). (This figure is available in color at *JXB* online.)

field and lab experiments examine whether root pressure and NSC variation contribute to recovery from desiccation.

Materials and methods

Plant material

We examined two DT fern species in the chaparral understorey of the Santa Monica Mountains (Los Angeles County, CA, USA). We selected these two species because they have contrasting frond size and shape, representing extremes in the frond morphology of DT fern species growing in the southern California chaparral (Figs 1, 3, 4). *Pentagramma triangularis* (formerly *Pentagramma triangularis* Kaulf. subsp. *triangularis*, see Schuettpelz *et al.*, 2015) has pinnate–pinnatifid fronds and short stature (generally <20 cm tall). In the desiccated state, the entire frond curls upward to form a single curled structure. *Pellaea andromedifolia* Kaulf. is two pinnate or three pinnate and ~50 cm tall. In the desiccated state, the smallest leaf segments (pinnules) curl downward, while the entire frond retains its open position. We used two similar study sites along the Backbone Trail of the Santa Monica Mountains for all field experiments and collections: the intersection of the Backbone Trail and Piuma Road (34°04′34″N, 118°41′10″W) and the Newton Canyon trailhead on Kanan Dume Road (34°04'34.0"N, 118°48'57.2"W). Our nomenclature is that of Baldwin *et al.* (2012) with modifications by Schuettpelz *et al.* (2015).

Experimental overview

Our study consisted of three experiments: a natural rain experiment *in situ* at the onset of the wet season, a root versus leaf irrigation experiment *in situ* during the dry season, and a water pressure versus capillary action experiment on stipes of excised fronds in an environmental chamber (Fig. 2). In the first experiment, we excluded rain water from the leaves of plants *in situ* during the first rain event of the wet season to test whether root water alone could resurrect the entire plant ('rain' experiment in Fig. 2A; *n*=4–5 plants per treatment). We compared desiccation recovery between the 'wet leaf' and 'dry leaf' treatments in this experiment. In the second experiment, we conducted *in situ* organ-specific irrigation experiments during the dry season to test whether leaf water alone could resurrect the entire

plant ('dry season' experiment in Fig. 2B; *n*=6 plants per treatment). We assessed which plant organs recovered when we watered the roots alone ('root irrigated') or leaves alone ('leaf irrigated'). In the last experiment, we applied water to the cut ends of excised fronds to test whether root pressure was needed for full recovery of the leaf tissue ('pressure' experiments in Fig. 2C; *n*=6 plants per treatment). Water was either forced into the cut end of the stipe using applied pressure ('30 kPa' in Fig. 2C) or applied to the cut end without pressure ('0 kPa' in Fig. 2C).

During resurrection, leaf tissue uncurls and photosynthetic capacity increases. We assessed increasing photosynthetic capacity by measuring dark-adapted chlorophyll fluorescence (F_v/F_m), which indicates the maximum efficiency of PSII and has previously been used as a measure of desiccation recovery (Proctor and Tuba, 2002; Watkins *et al.*, 2007; see also Maxwell and Johnson, 2000). Furthermore, we have found that F_v/F_m correlates with the maximum photosynthetic rate during resurrection in *P. triangularis* (Samantha Fiallo, unpublished data). Therefore, in our experiments, we quantified desiccation recovery in four ways, measuring (i) increase in dark-adapted chlorophyll fluorescence (F_v/F_m); (ii) increase in width of the frond or pinnule (leaflet) relative to the desiccated state; (iii) development of positive root pressure; and (iv) increase in water potential of the soil, stipe cortex, stipe vascular bundle, and leaf tissue. Plants were selected using haphazard sampling in the field. We selected fronds of similar size across individuals within species to avoid confounding effects of size on recovery. For both species, we selected a single representative frond on each plant before the experiment. Since recovery response was sometimes highly variable among individual fronds and pinnules, choosing fronds and pinnules *a priori* helped prevent sampling bias towards the resurrected pinnules. The leaf tissue on the selected frond was used to estimate the degree of recovery from desiccation once each day. For *P. andromedifolia*, two individual pinnules on the frond were chosen: one at the bottom and one at the top of the same frond. All pinnules chosen were located within 5 cm of the central rachis. The bottom pinnule was selected from the lowest branch on the frond. The top pinnule was selected within 5 cm of the frond tip.

The fronds are much smaller in *P. triangularis*, and the pinnae are often fused (Fig. 1). Unlike *P. andromedifolia*, the pinnae of *P. triangularis* are densely placed on the distal end of the frond and curl or uncurl as a unit while desiccating and resurrecting. Thus, only one measurement of F_v/F_m was taken per frond in *P. triangularis*.

Leaf recovery: F_v/F_m *and pinnule width*

We measured F_v/F_m and frond or pinnule width daily during all experiments using a pulse-modulated fluorometer and digital Vernier calipers (model OS1p, Opti Sciences, Hudson, NH, USA). In the field, dark adaptation clips or pieces of black cotton cloth were used to dark-adapt the leaf tissue for at least 20 min prior to F_v/F_m measurements, following the methods of Holmlund *et al.* (2016). We found that dark adaptation for a minimum of 20 min was sufficient for ferns to reach recovered F_v/F_m values above 0.8.

Stele recovery: F_v/F_m

Additionally, we measured the desiccation recovery of the photosynthetic tissue surrounding the vascular bundle (stele) inside the stipe using F_v/F_m (Fig. 4, inset photo). Only *P. andromedifolia* had a large enough stele for this measurement. The steles of *P. triangularis* also contain chlorenchyma; however, these steles were too small to reliably measure fluorescence. F_v/F_m of the stele was measured by carefully shaving away ~1 cm of the outer desiccated cortex (longitudinally) in the center third of the stipe (Fig. 4, inset photo). A stipe was selected that was not supplying water to the pinnules we were measuring (i.e. we used a different frond on the same plant). This exposed stele was wrapped in parafilm, and then dark adaptation clips were used to dark-adapt the stele as for the leaf tissue.

Root or rhizome recovery: root pressure

Preliminary field irrigation experiments on desiccated plants indicated that positive root pressures peaked ~2 d after irrigation began. Since a frond is sacrificed for each root pressure measurement, root pressure was measured in the morning only near the beginning of the rain experiment and near the beginning and end of the dry-season experiments using bubble manometers as in Ewers *et al.* (1997). Although we refer to the observed pressure as 'root pressure', our current methods do not allow us to distinguish between root pressure and rhizome pressure. Briefly, capillary tubes that had been sealed on one end were attached via flexible tubing to freshly cut stipes 2 cm above the soil. Before attaching the manometer to the plant, a bubble was inserted into the distal end of the manometer, and the rest of the tubing was filled with 20 mM KCl solution. Manometers were attached in the morning between 06.00 h and 09.00 h, and allowed to equilibrate 90 min before reading. The length of the bubble was measured before and after relieving the pressure by puncturing the tubing with a needle. This ratio was used to calculate root pressure using Equation 1:

$$P = 100[\left(\frac{L_{atm}}{L_{pressure}}\right) - 1] \qquad (1)$$

in which L_{atm} is the length of the bubble when the pressure is relieved and $L_{pressure}$ is the length of the bubble under root pressure (Ewers *et al.*, 1997). Manometers were not equilibrated overnight because changes in temperature caused additional small bubbles to form in the manometers, preventing an accurate reading. A small positive root pressure was observed in many of the desiccated unwatered controls (<3 kPa); however, this is probably an artifact of the method. It is likely that the lack of connection between the water in the bubble manometer and the embolized xylem conduits did not allow the manometer to fully equilibrate with desiccated plants, resulting in a slight positive pressure, which was generated when the manometer was attached to the stipe. This hypothesis is supported by our finding of slight positive pressures in desiccated control plants, which would have no water source to generate root pressure. Thus, root pressure values <3 kPa in all treatment groups were omitted from all analyses, since these values were likely to have been generated by this minor artifact.

Water potential

Water potential was measured at the end of the field experiments using a dew point hygrometer (WP4C Dewpoint PotentiaMeter, METER Group, Inc., Pullman, WA, USA). For *P. triangularis*, we measured soil and leaf water potential. For *P. andromedifolia*, we also measured the water potentials

of the steles and outer parenchyma (cortex) layers by quickly dissecting the stipes and rachises inside a humid bag before placing chopped samples in the dew point hygrometer (Fig. 4, inset). *Pentagramma triangularis* lacked sufficient stipe material to measure stele and cortex water potential.

Rainy-season experiments

To determine the contribution of leaf water to desiccation recovery, we took advantage of the first rain event of the wet season to conduct an experiment for 13 d to test the effects of leaf wetness on desiccation recovery (Fig. 2). On 7 January 2018, we covered all fronds of five individuals of each species with plastic bags to keep the leaves dry during a forecasted rain event. On 8–9 January 2018, our western study site received 69 mm of precipitation (obtained from Lechuza Patrol weather station, Los Angeles Department of Public Works). Prior to this rain event, all ferns at our field sites had been desiccated for ~7 months. Following the rain, we concluded that the roots of the plants were thoroughly soaked (based on excavations of some control plants), and all plants began to resurrect. All plastic bags were removed on the morning of 10 January, after the rain stopped. The effect of the plastic bags on the vapor pressure deficit (VPD) around the leaves was judged to be minimal since it was raining almost the entire time the bags were covering the plants.

No data were obtained on 9 January 2018 due to hazardous storm conditions. F_v/F_m and leaf width were measured daily from day 2 to day 12 following the rain event. Root pressure was measured in the morning near the beginning of the experiment (day 2). Tissue and soil water potentials were measured on day 13 post-rain. Some dew formed on *P. andromedifolia* leaves in the mornings of days 3 and 4. The effect of dew was judged to be minimal considering that all plants were affected equally, and that rain water on leaves should already have had an effect on leaves during the first 2 d.

Dry-season irrigation experiments

During the dry season, we conducted organ-specific field irrigation experiments for 16 d each to separately test the effects of root irrigation and leaf irrigation on the plants' resurrection response (Fig. 2). We used two types of irrigation. Root irrigation was achieved by daily watering the surrounding soil with 9.5 liters of water (days 0–15, total of 152 liters per plant over the course of 16 d; Ψ_{soil}= –0.05±0.00 MPa, –0.10±0.01 MPa, Table 1). Each plant was watered *in situ* with 9.5 liters daily in the late afternoon. Leaf irrigation was achieved by repeatedly spraying the leaves with de-ionized water during the day. De-ionized water was used to prevent accumulation of ions on the leaf surface following repeated spray treatments and evaporation. Due to high temperatures and low relative humidity, it was difficult to keep all the leaves wet even while repeatedly spraying them. Therefore, we covered the leaves with plastic bags to keep them wet. During the day, we removed the bags every 1–2 h to spray the leaves and prevent the build up of gases. We left the bags on the plants overnight to keep the leaves wet. The leaves were usually still wet in the morning, except for days 13–15 of the leaf irrigation experiment for *P. andromedifolia*, when the Santa Ana winds occurred at our study site, creating exceptionally hot, dry, and windy conditions. The bottom (proximal) pinnules occasionally dried out overnight since they were close to the bag opening. This difference in overnight wetting probably accounts for the difference in the recovery of top versus bottom pinnules in the leaf irrigation treatment of *P. andromedifolia* (Fig. 4I, J). The water potential of the soil surrounding the rhizomes of the leaf irrigation plants was thoroughly dry on day 16 of the experiment (–141±1 MPa, –117±5 MPa, Table 1), indicating that the leaf spraying had not hydrated the soil.

We measured F_v/F_m and pinnule width daily from day 0 (prior to watering) through to day 15. Root pressure was measured in the mornings on days 2 and 16 after starting irrigation. Samples for soil, stele, cortex, and leaf water potentials were collected at midday on day 16 after starting irrigation. In addition to the root irrigation and leaf irrigation treatments, we tracked 12 unmanipulated desiccated plants to confirm that plants remained desiccated throughout the experiment, not rehydrated by fog or dew. However, we did not include these plants in our statistical analyses.

Table 1. Tissue and soil water potential (in MPa) after resurrection *in situ* following rain, root irrigation, leaf irrigation, and a dry control group

	P. triangularis				P. andromedifolia			
	Rain	**Root irrigation**	**Leaf irrigation**	**Dry control**	**Rain**	**Root irrigation**	**Leaf irrigation**	**Dry control**
Ψ_{leaf}	−1.80±0.06 *−1.78±0.05*	−2.08±0.21	−0.88±0.10	−91.1±2.7	−1.92±0.07 *−2.13±0.07*	−1.96±0.06	−2.33±0.29	−77.3±7.4
Ψ_{stele}					−2.10±0.28 *−3.35±0.69*	−1.23±0.06	−19.4±9.2	−81.7±7.3
Ψ_{cortex}					−74.7±14.6 *−88.0±7.7*	−34.4±4.0	−46.0±6.7	−86.0±4.6
Ψ_{soil}	−0.05±0.02 *−0.07±0.03*	−0.05±0.00	−141.1±1.2	−100.3±10.4	−0.07±0.04 *−0.09±0.03*	−0.10±0.01	−117.3±5.1	−104.9±5.5

Data are means ±SE. Plants resurrected by the natural rain event belonged either to the dry leaf treatment group (italics) or to the wet leaf control group (Roman). No significant differences were observed in tissue or soil water potentials between wet leaf and dry leaf groups following rain (Student's *t*-test with equal variances, $P<0.05$). One outlier was removed from the *P. andromedifolia* leaf irrigation group (Ψ_{leaf}= −27.97 MPa); this leaf dried out in transit from field to lab.

Applied pressure experiments

In the lab, we performed applied pressure experiments under controlled conditions for 5 d to determine the relative contributions of root pressure and capillary action to whole-plant desiccation recovery (Fig. 2). To achieve this goal, we applied positive water pressure to the cut end of desiccated fronds in the lab under controlled conditions to mimic root pressures observed in plants resurrecting in the field. The resurrection response of these fronds was compared with the response of a control treatment subjected to capillary action alone (no pressure applied to the cut end of the stipe). Entire fronds were excised from the field while desiccated. Fronds were resurrected in a growth chamber (diurnal relative humidity=50–70%, temperature=20 °C daytime, 15 °C night, 14 h days). The relative humidity in our growth chamber fell within the range observed in the field (14–75%). Fans circulated air throughout the chamber to promote uniform conditions. Plant positions were rotated regularly. For the root pressure simulation, water pressure was applied to the cut end of the stipe using an elevated pressure head (30±0.5 kPa). For the control treatment, fronds were secured with the cut end submerged 1 cm deep in KCl solution in a 50 ml conical vial. F_v/F_m and frond/pinnule width were measured daily on the leaves on days 0 through 5. Prior to F_v/F_m measurements, plants were dark-adapted by turning off the lights for at least 20 min. In both capillary action and applied pressure treatments, we used filtered (0.1 μm filter) and degassed 20 mM KCl solution to slow bacterial and fungal growth in the xylem conduits. Additionally, all tubing and vials were bleached prior to the experiment.

Non-structural carbohydrate analyses in P. andromedifolia

NSC content was quantified in the natural rain experiment using the enzymatic methods of Lloret *et al.* (2018). NSC content was only examined in *P. andromedifolia* because individuals of *P. triangularis* were too small, lacking sufficient rhizome and stipe material for the analysis. Leaves of *P. triangularis* were not sampled because individual plants had too few leaves for repeated sampling. Briefly, rhizome, stele, and leaf tissue samples were collected from six *P. andromedifolia* individuals in the desiccated state (day 0) and on days 2, 5, and 8 after the natural rain event on 8–9 January 2018. Samples were transported on ice back to the lab, where they were microwaved for 30–60 s at 1500 W to stop enzymatic breakdown of sugars (i.e. consumption of NSC pools). Samples were dried in an oven at 60 °C to a constant mass and finely ground into a homogenous powder. An 11 mg aliquot of powder was dissolved in water and placed into a water bath at 80 °C to extract soluble sugars (i.e. sucrose, glucose, and fructose). We quantified soluble sugar by using enzymes to separately convert each soluble sugar to glucose-6-phosphate, which was then quantified optically at 340 nm using spectrophotometry. Total NSC content was separately determined by enzymatically digesting all NSCs into glucose-6-phosphate. Starch was then estimated as the difference between total NSCs and soluble sugars.

Statistical analyses

In the rain experiment, F_v/F_m and frond/pinnule width data were compared between wet leaf (rained) and dry leaf (bagged) treatments using a univariate repeated-measures ANOVA with a Greenhouse–Geisser correction factor to account for lack of sphericity (Figs 3–5). The same individuals were sampled repeatedly throughout the experiment, allowing the effect of time to be included in the analysis. *P*-values refer to the significance of the interaction between treatment and time effects (significant when $P<0.05$). The same analyses were used for the applied pressure versus capillarity experiment in the growth chamber (Fig. 6). In the rain experiment, root pressure and water potential data were compared between wet leaf and dry leaf treatments using a Student's *t*-test assuming equal variance ($P<0.05$, Figs 3, 4; Table 1). NSC data were analyzed using a one-way ANOVA within a given organ and sugar type followed by a Tukey's HSD post-hoc test ($P<0.05$, Figs 7, 8). Repeated-measures analyses were not appropriate for the NSC data because different individuals were sampled each time so that subsequent measurements would not be affected by previous rhizome excavations. All analyses were conducted using JMP Pro software (vs 14.2.0).

Results

Recovery following rain or dry-season irrigation

Following a 69 mm natural rain event on 8–9 January 2018, ferns rehydrated either naturally (wet leaf control) or with water excluded from the leaves covered by a bag (dry leaf treatment; Fig. 2). All individuals had been thoroughly desiccated for at least 7 months prior to this rain event. To further tease apart whether recovery could occur by either root water uptake alone or leaf water uptake alone, two additional treatments were implemented during the dry season using either daily root irrigation (days 0–15) or frequent leaf irrigation by spraying (Fig. 2).

Recovery of P. triangularis

Pentagramma triangularis is smaller than *P. andromedifolia* and responds differently to desiccation. As *P. triangularis* desiccates, water loss triggers inward curling of the pinnae, forming a cluster that exposes the yellow indument on the abaxial side of the leaf (Fig. 1A). The abaxial side of the leaf is presumably protected by the pale, waxy indument. Desiccation recovery
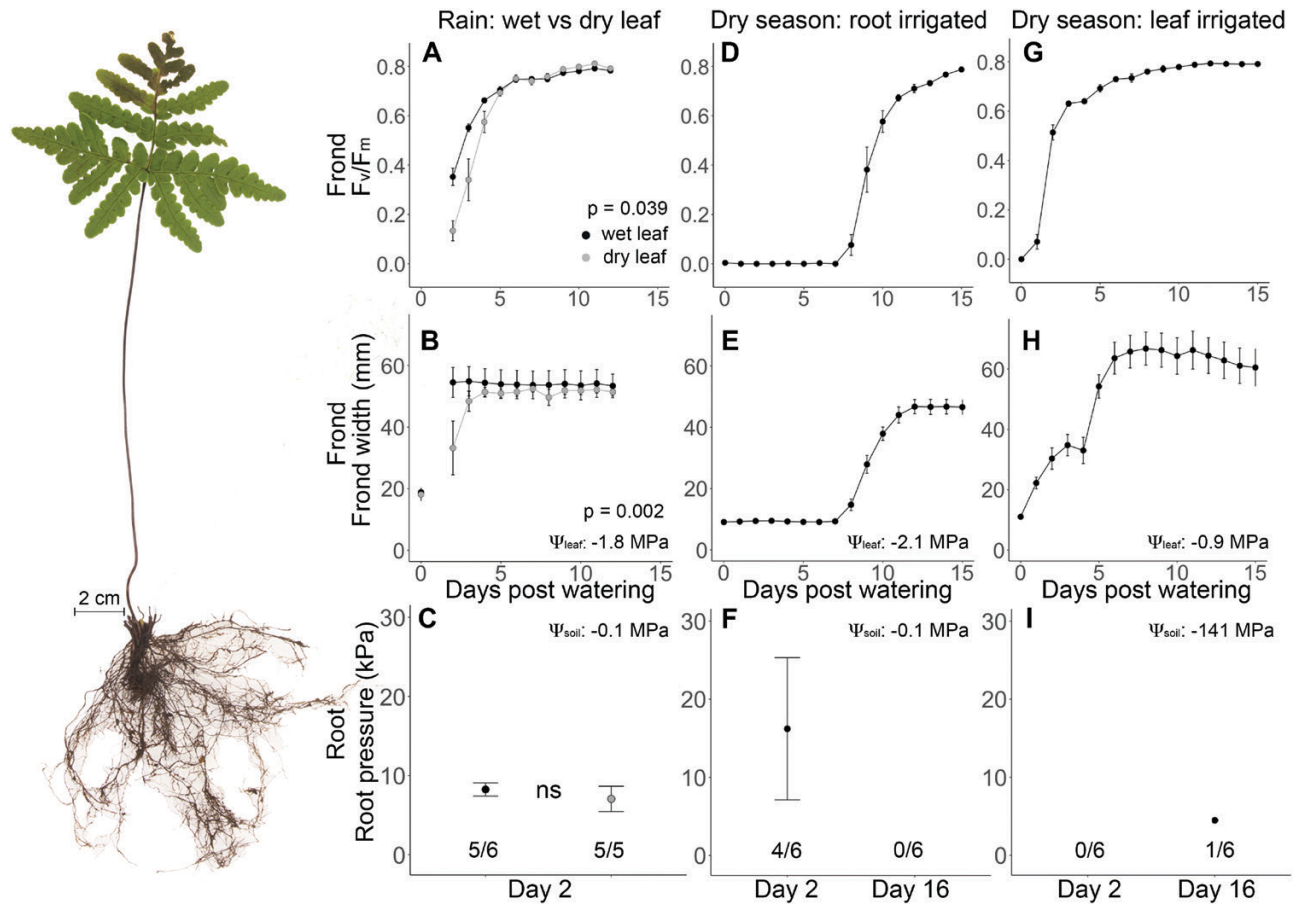
Fig. 3. Recovery of *Pentagramma triangularis* plants in the field following a natural rain event of 69 mm (A–C), root irrigation treatment during the dry season (D–F), and leaf irrigation treatment during the dry season (G–I). During the rain experiment (A–C), leaves were either allowed to be soaked by the rain (wet leaf control, black) or covered to keep them dry during the rain event (dry leaf treatment, gray). During recovery, fronds uncurl and regain photosynthetic capacity of PSII. We quantified recovery by measuring daily dark-adapted chlorophyll fluorescence, F_v/F_m (A, D, G), frond width (B, E, H), and root pressure on day 2 post-rehydration for the rain experiment (C) or days 2 and 16 for the organ-specific irrigation experiments in the dry season (F, I). Data shown are the mean ±SE, *n*=4–6. The proportions at the bottom indicate how many plants showed root pressure >3 kPa (C, F, I; see the Materials and methods). Post-rain *P*-values indicate the significance (*P*<0.05) of the interaction between time and treatment effects using a repeated-measures ANOVA with a G–G correction factor (F_v/F_m, frond width, significant) or a Student's *t*-test (root pressure, not significant). Water potential values (Ψ) indicate the water potential (MPa) of the leaf tissue or soil at the end of the experiment (*n*=6). (This figure is available in color at *JXB* online.)

was monitored in both the leaf tissue (uncurling frond) and the roots. After the rain, leaves in both the wet leaf (uncovered) and dry leaf (covered) groups uncurled and recovered healthy F_v/F_m (above 0.8) and frond width within 6 d, but the wet leaf group resurrected slightly faster than the dry leaf group (*P*=0.039, 0.002; Fig. 3A, B). However, this effect was localized to the leaf tissue, as there was no significant difference between the root pressure of the wet leaf and dry leaf groups on day 2 post rain (*P*=0.969; Fig. 3C).

During the dry season, the root irrigation treatment showed a similar but delayed resurrection response compared with both treatments in the rain experiment (Fig. 3D–F). The root irrigation treatment showed recovery in both roots and leaves, even though no water was applied directly to the leaves. The root-irrigated plants showed root pressure on day 2 post start of irrigation (16.2±9.1 kPa; Fig. 3F), consistent with root/rhizome recovery. However, leaf recovery was delayed until day 8 (Fig. 3D, E). In contrast, individuals in the leaf irrigation treatment only showed recovery in the leaves and not in the roots (Fig. 3G–I), indicating that no or insufficient water traveled from the leaves to the roots. No root pressure was observed

at the end of the dry-season irrigation experiment (day 16), with the exception of one plant in the leaf irrigation treatment showing slight pressure (4.5 kPa).

Recovery of *P. andromedifolia*

In contrast to *P. triangularis*, the desiccating fronds of *P. andromedifolia* maintain their open structure, while the terminal segments (pinnules) curl individually. In this species, the width of the individual pinnules was monitored instead of the width of the entire frond, since frond width remained approximately constant throughout recovery. Furthermore, *P. andromedifolia* is a larger plant, allowing recovery of the stele to be monitored as well as that of the leaves and roots. In the stipes, recovery of the inner vascular system (stele, including the endodermis and pericycle surrounding the vascular tissue) was monitored by measuring F_v/F_m because the stele contained chlorenchyma surrounding the phloem and xylem (Fig. 4, inset photo).

Following the natural rain event, the stele chlorenchyma of the stipe of both wet leaf and dry leaf plants recovered F_v/F_m in a manner similar to the leaf tissue (Fig. 4C). Individuals with either
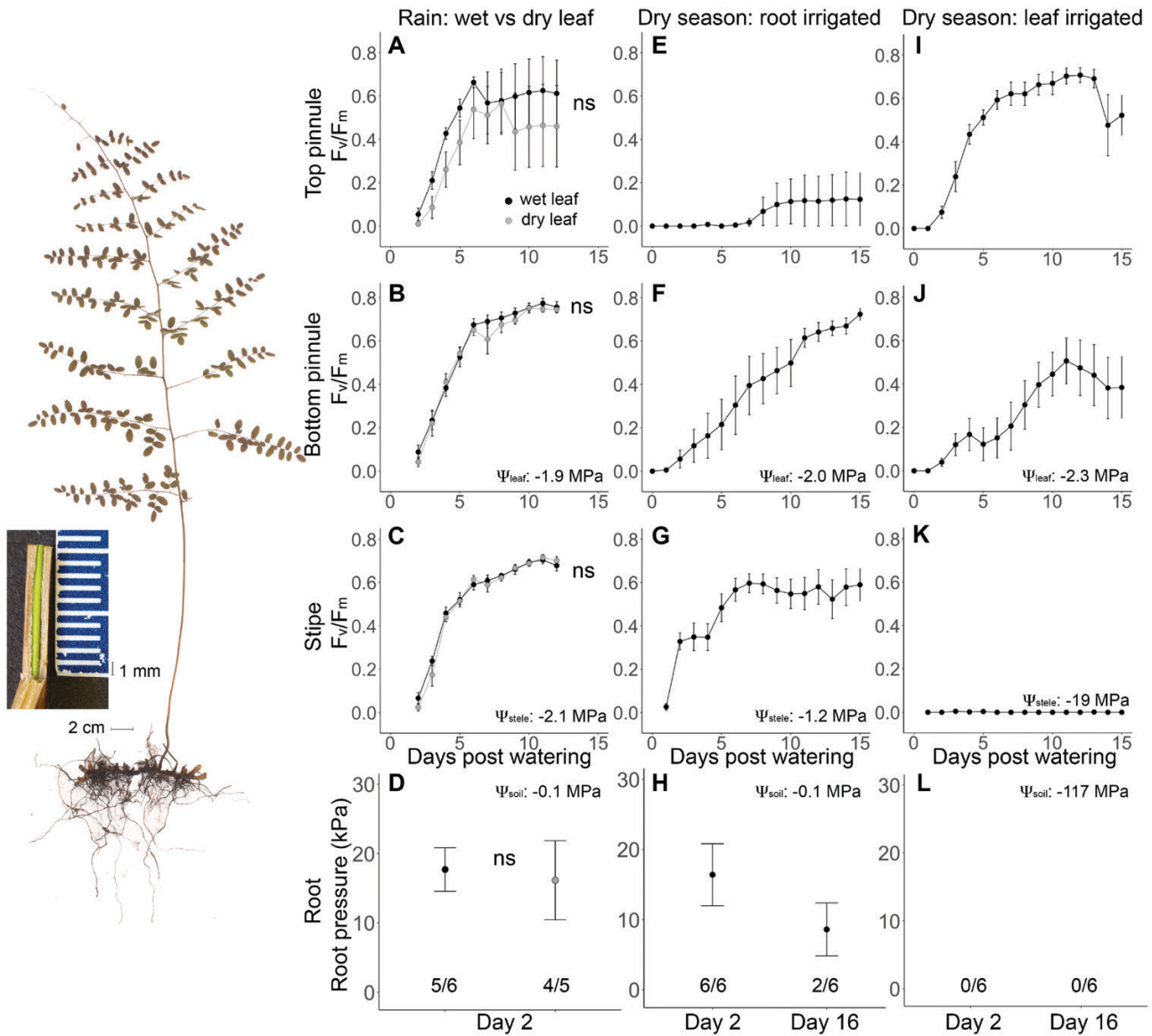
Fig. 4. Recovery of *Pellaea andromedifolia* plants in the field following a natural rain event (69 mm, A–D), root irrigation treatment during the dry season (E–H), and leaf irrigation treatment during the dry season (I–L). During the rain experiment (A–D), leaves were either allowed to be soaked by the rain (wet leaf control, black) or covered to keep them dry during the rain event (dry leaf treatment, gray). We quantified recovery by measuring daily dark-adapted chlorophyll fluorescence, F_v/F_m (A–C, E–G, I–K), and root pressure on day 2 post-rehydration for the rain experiment (D) or days 2 and 16 for the organ-specific irrigation experiments in the dry season (H, L). Data shown are the mean ± SE, n=5–6. The proportions at the bottom indicate how many plants showed root pressure >3 kPa (D, H, L; see the Materials and methods). Post-rain P-values indicate the significance (P<0.05) of the interaction between time and treatment effects using a repeated-measures ANOVA with a G–G correction factor (F_v/F_m, not significant) or a Student's t-test (root pressure, not significant). Water potential values (Ψ) indicate the water potential (MPa) of the leaf, stele, or soil at the end of the experiment (n=5–6). (This figure is available in color at *JXB* online.)

wet (uncovered) or dry (covered) leaves recovered F_v/F_m equally well in the top pinnule (Fig. 4A; P=0.713), bottom pinnule (Fig. 4B; P=0.548), stele (Fig. 4C; P=0.261), and roots/rhizome (Fig. 4D; P=0.819). The F_v/F_m values above 0.8 in recovered tissues were consistent with the hydrated tissue and soil water potentials at the end of the experiment (day 13 post rain) (Fig. 4; Table 1).

In the dry-season root irrigation treatment, all plant organs (root/rhizome, stele, leaf) improved F_v/F_m even though water was only applied to the roots (Fig. 4E–H); however, the water did not reach topmost pinnules during the course of the experiment (Fig. 4E). This result contrasted with the recovery observed in the dry leaf treatment from the natural rain experiment, in which only the roots received water and yet all

pinnules recovered (including the top pinnules). This difference is probably due to the high temperatures and low relative humidity during the dry season, which probably slowed recovery of the dry-season root-irrigated plants. The soil surrounding the roots was thoroughly hydrated in both experiments (Ψ$_{soil}$≥ −0.10 MPa, Table 1). Soil and tissue water potentials of root-irrigated plants at the end of the dry-season experiment showed a clear gradient from the soil near the roots (−0.1 MPa) to the stele (−1.2 MPa) to resurrected leaf tissue (−2.0 MPa) (Fig. 4B–D; Table 1). The stipe cortex tissue appeared to be hydraulically isolated from the neighboring stele tissue (−34.4±4.0 MPa versus −1.23±0.06 MPa) and showed no signs of recovery (Table 1; Fig. 4, inset). The root-irrigated
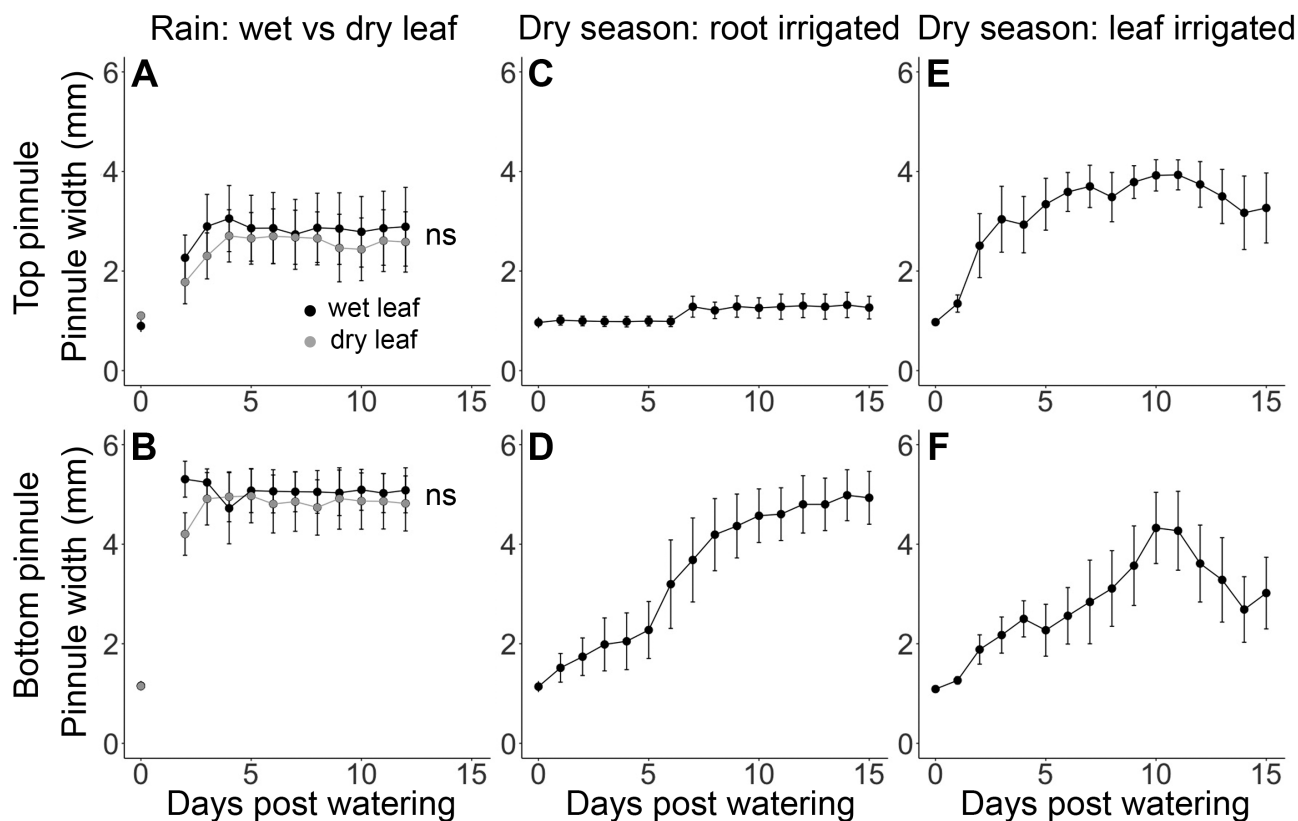
Fig. 5. Recovery of *Pellaea andromedifolia* plants in the field following a natural rain event (69 mm, A, B), root irrigation treatment during the dry season (C, D), and leaf irrigation treatment during the dry season (E, F). During the rain experiment, leaves were either allowed to be soaked by the rain (wet leaf control, black) or covered to keep them dry during the rain event (dry leaf treatment, gray). We quantified recovery by measuring daily pinnule width with digital Vernier calipers. Data shown are the mean ±SE, *n*=5–6. Post-rain *P*-values indicate the significance (*P*<0.05) of the interaction between time and treatments effects using a repeated-measures ANOVA with a G–G correction factor (not significant).

plants showed root pressure on day 2 post start of irrigation (16.4±4.4 kPa; Fig. 4H). Most root pressure had dissipated by the end of the dry-season irrigation experiments; only two plants in the root irrigation treatment showed root pressure on day 16 (12.4 kPa and 4.8 kPa). These data are consistent with the finding that root pressure dissipated by day 6 following the natural rain event in the wet season (data not shown).

In the leaf-irrigated plants from the dry-season experiment, only leaf tissue resurrected; the stele and roots remained desiccated (Fig. 4I–L). Top pinnules resurrected more quickly and more completely (higher F_v/F_m and leaf width) than bottom pinnules, perhaps due to more thorough overnight wetting farther inside the bag (Fig. 4I, J), or perhaps because the bottom pinnules were losing more water to the stele. Pinnule expansion closely followed F_v/F_m recovery in the leaf tissue (Fig. 5). However, steles in the leaf irrigation treatment showed no signs of F_v/F_m recovery, and this result is consistent with their dehydrated status on day 16 of the experiment (−19.4±9.2 MPa; Fig. 4K; Table 1). No root/rhizome recovery was observed in the leaf irrigation treatment on days 2 or 16 (Fig. 4L).

Applied pressure experiments

Given that root water uptake appeared to be critical for whole-plant desiccation recovery, this subsequent experiment tested whether the root pressure observed in intact, rehydrating plants

in situ was actually necessary for desiccation recovery, or if capillary action alone inside xylem conduits can resurrect the stele and leaves. We rehydrated excised fronds in a growth chamber either by applying 30 kPa of water pressure to the cut end of the stipe (simulated root pressure) or by placing the cut end of the stipe in water (capillary action alone) (Fig. 2). F_v/F_m of the bottom pinnules of *P. andromedifolia* recovered equally well with capillary action alone and with simulated root pressure (Fig. 6C, NS). However, the leaf tissue of *P. triangularis* and the top pinnules of *P. andromedifolia* showed improved recovery when pressure was applied to the cut end of the stipe compared with capillary action alone (Fig. 6A, B, *P*=0.014, 0.004). Frond or pinnule width closely followed F_v/F_m in these experiments; however, there was high variability in pinnule size (Fig. 6D–F). Changes in frond width of *P. triangularis* were marginally significant between treatments (Fig. 6D; *P*=0.051). Pinnule width of *P. andromedifolia* increased more rapidly with applied pressure for the top pinnule (Fig. 6E; *P*=0.022) and was not significantly different between treatments for the bottom pinnule (Fig. 6F; NS).

Non-structural carbohydrate analysis in P. andromedifolia

NSC content was analyzed enzymatically in the leaves, steles, and rhizomes of resurrecting *P. andromedifolia* individuals following the 69 mm rain event on 8–9 January 2018 (i.e. natural
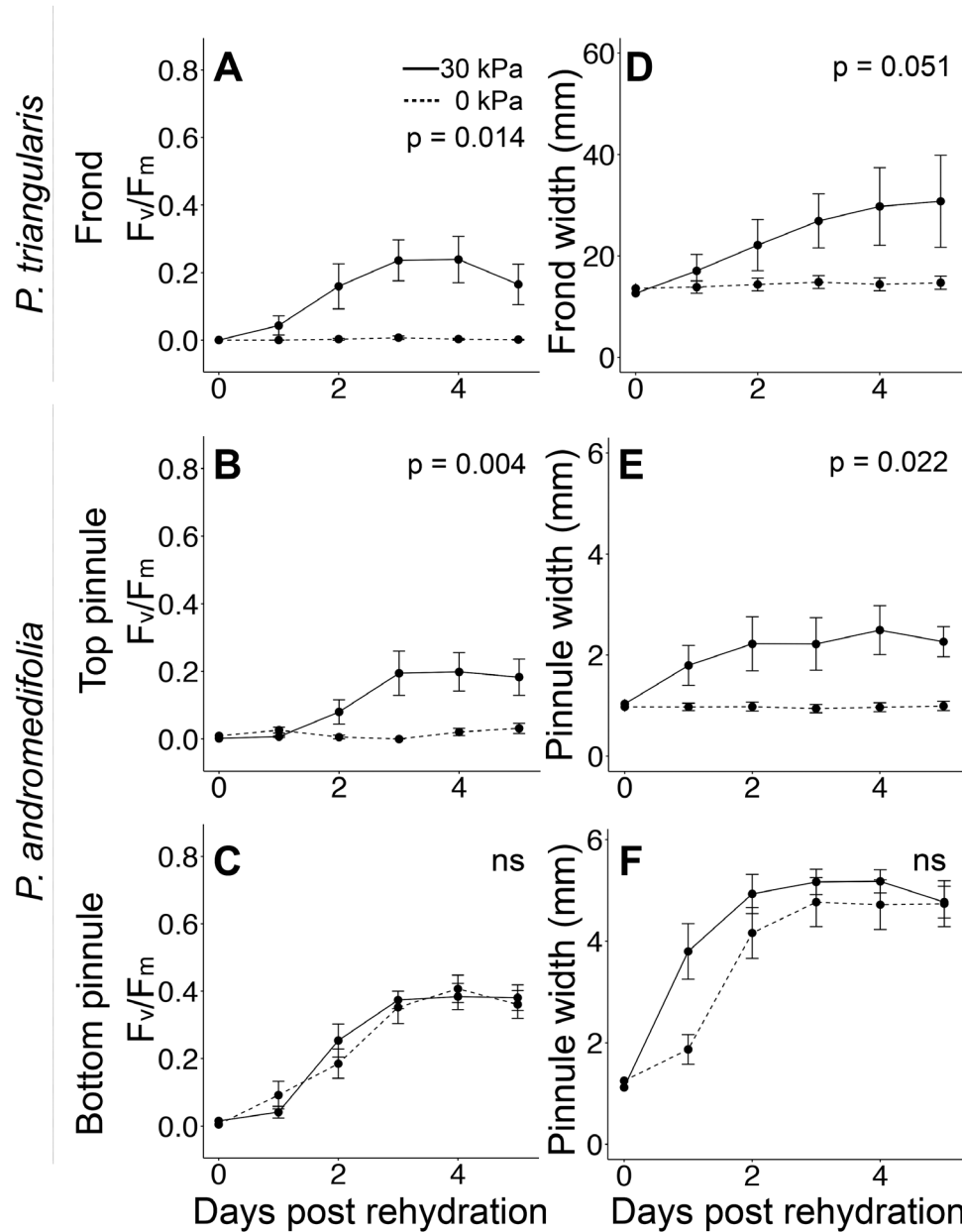
Fig. 6. (A, D) Recovery of F_v/F_m and frond width (mm) in excised fronds of *Pentagramma triangularis*. (B, C, E, F). Recovery of F_v/F_m and pinnule width at the top and bottom of *Pellaea andromedifolia* fronds The excised fronds were subjected either to pressure applied to the cut end of the frond (30 kPa, solid line) or capillary action alone (0 kPa, dashed line). At no time was liquid water applied to fronds or pinnules to promote foliar water uptake. Data are the mean ±SE, *n*=6. For F_v/F_m data, there was no significant difference between pressure and non-pressure treatments for the bottom pinnules of *P. andromedifolia*, but there was an overall difference between treatments with time for the top pinnules of *P. andromedifolia* (*P*=0.004) and for the fronds of *P. triangularis* (*P*=0.014). Consistent with this observation, there were no significant differences in pinnule width between treatments in the bottom pinnules of *P. andromedifolia*, but there was a significant difference for top pinnules of *P. andromedifolia* (*P*=0.022) and a marginally significant differences for the fronds of *P. triangularis* (*P*=0.051). *P*-values indicate the significance of the interaction between time and treatment effects using a repeated-measures ANOVA with a G–G correction factor.

rain experiment). Insufficient material was available to measure NSC content in *P. triangularis* due to the small size and number of leaves on each plant. Desiccated plant tissue was collected before the rain, and resurrecting plant tissue was collected 2, 5, and 8 d after the rain. Total NSC content did not change over time in the leaf tissue and rhizomes of recovering individuals, but NSC content decreased in the steles during the resurrection process (*P*<0.05; Fig. 7). However, separate analyses of starch, sucrose, and glucose/fructose content revealed more complex patterns (Fig. 8). Further analysis indicated that leaf tissue showed no change in any of the three NSC components (starch, sucrose, and glucose/fructose) over time (*P*=0.36, 0.76, 0.97; Fig. 8A). In contrast, both the steles and rhizomes showed decreased sucrose in the resurrected state compared with the desiccated state (*P*<0.05; Fig. 8B, C). Both the steles and rhizomes showed a slight peak in glucose/fructose content on day 2 post rain (Fig. 8B, C). Starch was absent from both the steles and the rhizomes in the desiccated state; however, starch rose steadily in the rhizome throughout the recovery process (*P*<0.05; Fig. 8C).
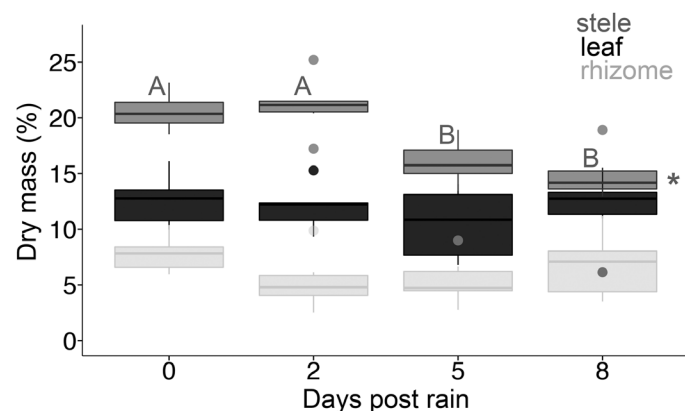
Fig. 7. Total non-structural carbohydrate content by organ in recovering individuals of *P. andromedifolia* immediately before (day 0) and following (days 2, 5, and 8) a natural rain event of 69 mm on 8–9 January 2018. Asterisks and letters indicate a significant difference among days within an organ (*P*<0.05) as determined by a one-way ANOVA followed by a Tukey's HSD post-hoc test. Boxplots represent the first and third quartile, median, and 95% confidence intervals, with outliers shown (*n*=6). Plants were not repeatedly sampled to avoid artifacts from damaged individuals. Thus, different plants were sampled (*n*=6) from the population each day.

Discussion

Our results show that three factors contribute to desiccation recovery in *P. andromedifolia* and *P. triangularis*: root pressure, capillary action, and foliar water uptake. Experimentally excluding natural rain from a desiccated leaf showed that a combination of root pressure and capillary action resurrected all plant organs (root, stipe, and leaf) without the aid of foliar water uptake. Root irrigation during the dry season showed that positive hydraulic root pressure and capillary action resurrected all plant organs, although some distal pinnules never recovered, possibly due to the higher VPD in the dry season. However, leaf recovery happened most rapidly via foliar water uptake, as in the dry season leaf irrigation experiment and the wet leaf treatment in the natural rain experiment. Therefore, desiccation recovery in natural conditions probably involves all three mechanisms.

However, root pressure is likely to play a critical role in recovering the stipe. Stipe F_v/F_m in *P. andromedifolia* only recovered when water was applied to the roots; foliar water uptake did not rehydrate the stipe despite 15 d of wetting the leaves (Fig. 4K). In the chamber experiment, capillary action succeeded in resurrecting proximal pinnules in *P. andromedifolia*, but applied water pressure was needed to resurrect distal pinnules in *P. andromedifolia* and the fronds of *P. triangularis* (Fig. 6). Thus, root pressure probably drives stipe recovery (including xylem embolism repair) in natural conditions. Indeed, our previous study found that gas embolism is repaired in stipe xylem early in the resurrection process when only the roots were watered (Holmlund *et al.*, 2019). Restoring hydraulic flow through the vascular system would be essential for long-term water supply to the leaf blades and pinnules. Without stipe hydraulic flow, the leaf blades and pinnules would probably desiccate after the leaf surface water evaporated or was absorbed.

Further experiments are needed to determine whether a combination of capillary action and foliar water uptake could
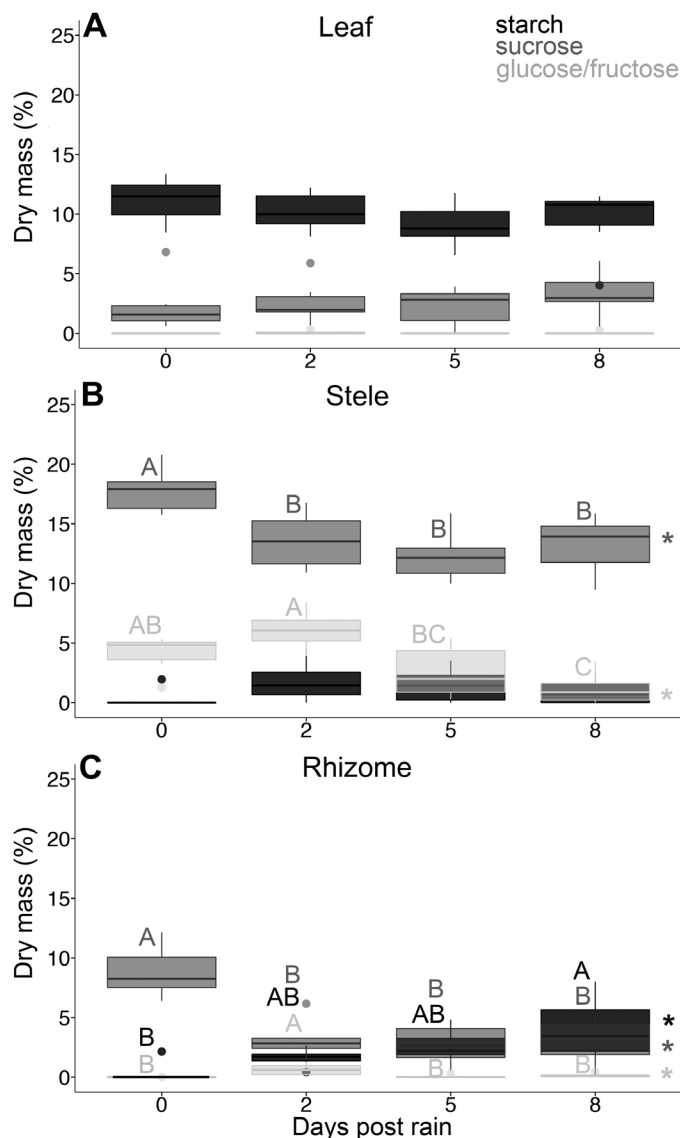
Fig. 8. Non-structural carbohydrate content by sugar type in recovering individuals of *P. andromedifolia* immediately before (day 0) and following (days 2, 5, and 8) a natural rain event of 69 mm on 8–9 January 2018. Data are shown for the leaf tissue (A), stele (B), and rhizomes (C) for starch content (black), sucrose content (dark gray), and combined glucose and fructose content (light gray). Asterisks and letters indicate a significant difference among days within a given organ and sugar type (*P*<0.05) as determined by a one-way ANOVA followed by a Tukey's HSD post-hoc test. Boxplots represent the first and third quartile, median, and 95% confidence intervals, with outliers shown (*n*=6). Plants were not repeatedly sampled to avoid artifacts from damaged individuals. Thus, different plants were sampled (*n*=6) from the population each day.

enable whole-plant recovery without the aid of root pressure. However, this scenario is probably not representative of natural conditions, given that root pressure was nearly always observed whenever water was applied to the roots (Figs 3C, F, 4D, H). The data presented here suggest that root pressure probably initiates whole-plant recovery by rehydrating the vascular system while foliar water uptake concurrently expedites leaf recovery.

Root pressure has been shown to occur in non-DT ferns (Sperry, 1983; Fisher *et al.*, 1997), but the role of root pressure in desiccation recovery in DT ferns was previously unknown. *Pleopeltis polypodioides*, a subtropical epiphyte, is perhaps the

most commonly studied DT fern, but we are unaware of any reports of root pressure in this species. Instead, previous studies have shown that *P. polypodioides* relies on specialized scales for leaf water uptake (John and Hasenstein, 2017). Our study on chaparral ferns may provide an interesting contrast to previous work on *P. polypodioides*, since these two groups of ferns may have re-evolved different desiccation tolerance mechanisms to fill their respective niches. Resurrection fern species may have evolved diverse strategies for tolerating desiccation because desiccation tolerance in the vegetative tissues has apparently re-evolved in vascular plants (Gaff and Oliver, 2013; VanBuren *et al.*, 2017). Root pressure may help terrestrial ferns take full advantage of a timely seasonal rain event, whereas subtropical epiphytic ferns may capture sufficient water through foliar water uptake following frequent rain events.

Previous studies on the DT angiosperm *Myrothamnus flabellifolius* have produced conflicting reports regarding the role of root pressure in angiosperm desiccation recovery. Schneider *et al.* (2000) found that root pressure was required to remove a lipid layer lining the tracheary elements in *M. flabellifolius*. Sherwin *et al.* (1998) found that capillary action, and not root pressure, was likely to be responsible for whole-plant recovery, since they only observed 2.4 kPa of root pressure in resurrecting plants. Evidence was found for capillary refilling, and excised stems even resurrected under −8 kPa of tension (Sherwin *et al.*, 1998). However, root pressure generation may have been hindered by the fact that the plants in that study were potted, because subsequent studies found up to 13 kPa root pressure for this species *in situ* (Canny, 2000). Although no one has directly compared hydraulic recovery in DT ferns and DT angiosperms, some ferns lacking specialized structures for foliar water uptake may require root pressure for resurrection even if angiosperms do not. Ferns have tracheid-based xylem with less xylem per unit stipe transverse area, which contributes to higher xylem resistance per unit stipe transverse area than those of angiosperms and gymnosperms (Pittermann *et al.*, 2011, 2013). Reduced xylem transverse area in ferns might hinder rapid resurrection in the absence of root pressure, at least for the most distal leaf tissue.

Although root pressure is critical for whole-plant recovery in these two DT fern species, many other factors probably affect the speed and success of desiccation recovery. High ambient temperature and low relative humidity (high VPD) are likely to have slowed desiccation recovery during the dry-season root irrigation experiment, even though the soil was fully hydrated in both the dry-season root irrigation experiment and the natural rain experiment (Table 1). Warmer and drier atmospheric conditions probably cause more water to evaporate from the liquid to the gas phase in the stipe xylem conduits of resurrecting plants, slowing the movement of liquid water to the leaf blades. Reduced path length and xylem resistance might explain why the bottom (proximal) pinnules of *P. andromedifolia* resurrected with capillary action alone in the chamber experiment while the top (distal) pinnules required applied pressure to resurrect. However, the low VPD experienced by the plants during the natural winter rain experiment is perhaps more typical of the conditions usually experienced during resurrection in the Santa Monica Mountains. It is possible that root pressure would be less critical for whole-plant recovery if high atmospheric moisture (low VPD) was maintained until all plant organs had resurrected, although this hypothesis was not tested in our study.

Our study of the NSC dynamics in three organs of resurrecting *P. andromedifolia* may provide some insight into the mechanism of desiccation protection in this chaparral DT fern species. Sucrose was concentrated in some of the desiccated tissues, especially the stele (Fig. 8B). These data are consistent with other studies identifying sucrose as a desiccation protectant in other DT species (reviewed in Scott, 2000). The photosynthetic stele (Fig. 4, inset) showed the highest NSC content throughout the experiment, perhaps due to the densely packed chloroplasts surrounding the xylem and phloem. Notably, the three organs showed different NSC dynamics. Sucrose and starch fluctuated in the rhizome. During recovery, sucrose content dropped rapidly while starch content increased steadily. The rhizome sucrose may have been converted into starch for long-term storage, perhaps as a resource for the next desiccation event. The progressive storage of starch is consistent with the role of rhizomes in carbon storage (Martínez-Vilalta *et al.*, 2016). Conversion to starch may also maximize assimilation by preventing build up of the product (sucrose). Sucrose in the stele also decreased on day 2 post rain, and glucose and fructose decreased by day 5 post rain. It is possible that these sugars in the stipe may have declined after first providing energy for xylem embolism repair and possibly solutes for the rapid build up of positive hydrostatic pressure in the stipe, although our study did not directly test either of those hypotheses. Previous studies have suggested that sucrose may facilitate embolism reversal, even in the absence of root pressure (Secchi and Zwieniecki, 2011; Secchi and Zwieniecki, 2012; Savi *et al.*, 2016). Furthermore, Schmitz *et al.* (2012) found a correlation between hydraulic conductivity and light availability to xylary chloroplasts in a non-DT angiosperm, implying that xylary chloroplasts may facilitate embolism repair. Although our study did not directly test the role of NSCs or xylary chloroplasts in desiccation recovery, this concept may be an interesting direction for future study, especially in DT species lacking root pressure.

Holmlund *et al.* (2016) speculated that DT ferns would fare better than other fern species in the future as climate change leads to longer and more severe droughts in southern California. The results from this study may provide support for this hypothesis, since the first seasonal rain event came unusually late in January 2018 and still the ferns resurrected rapidly and fully. These results, combined with future studies, will elucidate the mechanisms of desiccation recovery on a whole-plant scale, thus providing insight into the minimum survival requirements of the resurrection ferns.

Acknowledgements

References

Alcantara S, de Mello-Silva R, Teodoro GS, Drequeceler K, Ackerly DD, Oliveira RS. 2015. Carbon assimilation and habitat segregation in resurrection plants: a comparison between desiccation- and non-desiccation-tolerant species of Neotropical Velloziaceae (Pandanales). Functional Ecology **29**, 1499–1512.

Alpert P. 2005. The limits and frontiers of desiccation-tolerant life. Integrative and Comparative Biology **45**, 685–695.

Baldwin BG, Goldman DH, Keil DJ, Patterson R, Rosatti TJ, Wilken DH. eds. 2012. The Jepson manual: vascular plants of California. Berkeley, CA: University of California Press.

Canny MJ. 2000. Water transport at the extreme—restoring the hydraulic system in a resurrection plant. New Phytologist **148**, 187–189.

Cowling RM, Rundel PW, Lamont BB, Kalin Arroyo M, Arianoutsou M. 1996. Plant diversity in mediterranean-climate regions. Trends in Ecology & Evolution **11**, 362–366.

Ewers FW, Cochard H, Tyree MT. 1997. A survey of root pressures in vines of a tropical lowland forest. Oecologia **110**, 191–196.

Farrant JM, Brandt W, Lindsey GG. 2007. An overview of mechanisms of desiccation tolerance in selected angiosperm resurrection plants. Plant Stress **1**, 72–84.

Fisher JB, Guillermo Angeles A, Ewers FW, Lopez-Portillo J. 1997. Survey of root pressure in tropical vines and woody species. International Journal of Plant Sciences **158**, 44–50.

Gaff DF. 1977. Desiccation tolerant vascular plants of southern Africa. Oecologia **31**, 95–109.

Gaff DF. 1987. Desiccation tolerant plants in South America. Oecologia **74**, 133–136.

Gaff DF, Oliver M. 2013. The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon. Functional Plant Biology **40**, 315–428.

Holmlund HI, Lekson VM, Gillespie BM, Nakamatsu NA, Burns AM, Sauer KE, Pittermann J, Davis SD. 2016. Seasonal changes in tissue-water relations for eight species of ferns during historic drought in California. American Journal of Botany **103**, 1607–1617.

Holmlund HI, Pratt RB, Jacobsen AL, Davis SD, Pittermann J. 2019. High-resolution computed tomography reveals dynamics of desiccation and rehydration in fern petioles of a desiccation-tolerant fern. New Phytologist **224**, 97–105.

John SP, Hasenstein KH. 2017. The role of peltate scales in desiccation tolerance of *Pleopeltis polypodioides.* Planta **245**, 207–220.

Lloret F, Sapes G, Rosas T, Galiano L, Saura-Mas S, Sala A, Martínez-Vilalta J. 2018. Non-structural carbohydrate dynamics associated with drought-induced die-off in woody species of a shrubland community. Annals of Botany **121**, 1383–1396.

Martínez-Vilalta J, Sala A, Asensio D, Galiano L, Hoch G, Palacio S, Piper FI, Lloret F. 2016. Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. Ecological Monographs **86**, 495–516.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence—a practical guide. Journal of Experimental Botany **51**, 659–668.

Moore JP, Hearshaw M, Ravenscroft N, Lindsey GG, Farrant JM, Brandt WF. 2007. Desiccation-induced ultrastructural and biochemical changes in the leaves of the resurrection plant *Myrothamnus flabellifolia*. Australian Journal of Botany **55**, 482–491

Oliver MJ, Tuba Z, Mishler BD. 2000. The evolution of vegetative desiccation tolerance in land plants. Plant Ecology **151**, 85–100.

Peters S, Mundree SG, Thomson JA, Farrant JM, Keller F. 2007. Protection mechanisms in the resurrection plant *Xerophyta viscosa* (Baker): both sucrose and raffinose family oligosaccharides (RFOs) accumulate in leaves in response to water deficit. Journal of Experimental Botany **58**, 1947–1956.

Pittermann J, Brodersen C, Watkins JE Jr. 2013. The physiological resilience of fern sporophytes and gametophytes: advances in water relations offer new insights into an old lineage. Frontiers in Plant Science **4**, 285.

Pittermann J, Limm E, Rico C, Christman MA. 2011. Structure–function constraints of tracheid-based xylem: a comparison of conifers and ferns. New Phytologist **192**, 449–461.

Porembski S, Barthlott W. 2000. Granitic and gneissic outcrops (inselbergs) as centers of diversity for desiccation-tolerant vascular plants. Plant Ecology **151**, 19–28.

Proctor MC. 2000. The bryophyte paradox: tolerance of desiccation, evasion of drought. Plant Ecology **151**, 41–49.

Proctor MC, Tuba Z. 2002. Poikilohydry and homoihydry: antithesis or spectrum of possibilities? New Phytologist **156**, 327–349.

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD. 2007. Desiccation-tolerance in bryophytes: a review. The Bryologist **110**, 595–621.

Savi T, Casolo V, Luglio J, Bertuzzi S, Lo Gullo MA, Nardini A. 2016. Species-specific reversal of stem xylem embolism after a prolonged drought correlates to endpoint concentration of soluble sugars. Plant Physiology and Biochemistry **106**, 198–207.

Schmitz N, Egerton JJ, Lovelock CE, Ball MC. 2012. Light-dependent maintenance of hydraulic function in mangrove branches: do xylary chloroplasts play a role in embolism repair? New Phytologist **195**, 40–46.

Schneider H, Wistuba N, Wagner HJ, Thurmer F, Zimmermann U. 2000. Water rise kinetics in refilling xylem after desiccation in a resurrection plant. New Phytologist **148**, 221–238.

Schuettpelz E, Pryer KM, Windham MD. 2015. A unified approach to taxonomic delimitation in the fern genus *Pentagramma* (Pteridaceae). Systematic Botany **40**, 629–644.

Scott P. 2000. Resurrection plants and the secrets of eternal leaf. Annals of Botany **85**, 159–166.

Secchi F, Zwieniecki MA. 2011. Sensing embolism in xylem vessels: the role of sucrose as a trigger for refilling. Plant, Cell & Environment **34**, 514–524.

Secchi F, Zwieniecki MA. 2012. Analysis of xylem sap from functional (nonembolized) and nonfunctional (embolized) vessels of *Populus nigra*: chemistry of refilling. Plant Physiology **160**, 955–964.

Sherwin HW, Pammenter N, February E, Willigen CV, Farrant JM. 1998. Xylem hydraulic characteristics, water relations and wood anatomy of the resurrection plant *Myrothamnus flabellifolius* Welw. Annals of Botany **81**, 567–575.

Sperry JS. 1983. Observations on the structure and function of hydathodes in *Blechnum lehmannii*. American Fern Journal **73**, 65–72.

VanBuren R, Wai CM, Zhang Q, Song X, Edger PP, Bryant D, Michael TP, Mockler TC, Bartels D. 2017. Seed desiccation mechanisms co-opted for vegetative desiccation in the resurrection grass *Oropetium thomaeum*. Plant, Cell & Environment **40**, 2292–2306.

Watkins JE Jr, Mack MC, Sinclair TR, Mulkey SS. 2007. Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. New Phytologist **176**, 708–717.